18/D

IMPERIAL MYCOLOGICAL INSTITUTE
6 AUG. 1935

THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY

Vol. 21, N.S., Nos. 22–26. JULY, 1935.

22.—INVESTIGATIONS ON THE CONTROL OF SEEDLING DISEASES OF SUGAR BEET. By WILLIAM HUGHES, B.AGR.SC., M.SC., Albert Agricultural College, Glasnevin, Dublin. [Communicated by DR. P. A. MURPHY.]

23.—ON THE CHARACTERISTICS OF *BACTERIUM VIOLACEUM* (SCHRÖTER) AND SOME ALLIED SPECIES OF VIOLET BACTERIA. By GEORGE CRUESS-CALLAGHAN, M.SC., and M. J. GORMAN, A.R.C.SC.I. (Plate 6.)

24.—A SIMPLE TITRAMETRIC METHOD FOR THE APPROXIMATE DETERMINATION OF MILK PHOSPHATES. By G. T. PYNE, Department of Dairy Chemistry, University College, Cork. [Communicated by PROFESSOR T. J. NOLAN.]

25.—REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1934. (Including Reports by OLIVER CHANCE, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Coombe, and St. Anne's Hospitals; W. G. HARVEY, M.D., F.R.C.P.I., Adelaide Hospital, Dublin; OSWALD J. MURPHY, M.B., B.CH., St. Vincent's Hospital, Dublin.

26.—NOTE ON THE EFFECT OF STORAGE ON THE COLOUR AND ON THE FREE FATTY ACID CONTENT OF A COMMERCIAL SAMPLE OF VETERINARY COD-LIVER OIL. By E. J. SHEEHY, F.R.C.SC.I., B.SC., University College, Dublin.

[*Authors alone are responsible for all opinions expressed in their Communications.*]

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,

BALLSBRIDGE, DUBLIN.

HODGES, FIGGIS, AND CO.,

20 NASSAU STREET, DUBLIN.

WILLIAMS AND NORGATE,

28 TO 30 LITTLE RUSSELL STREET, STRAND, LONDON, W.C. 2.

1935.

Price Three Shillings.

Royal Dublin Society.

FOUNDED, A.D. 1731. INCORPORATED, A.D. 1750.

The Scientific Meetings of the Society are usually held at 4.15 p.m. on the fourth Tuesday of every month of the Session (November to June).

The attention of authors is directed to the following extracts from the regulations governing the presentation of communications at Scientific Meetings of the Society, and the publication of papers in the Scientific or Economic Proceedings:—

> "An author who desires to present a communication at a Scientific Meeting of the Society shall furnish to the Registrar a fortnight previous to that meeting the title and an abstract (not exceeding 200 words) of his communication, together with an estimate of the time required for presentation.
>
> "An author who desires to have a communication published forthwith as a paper in the Proceedings of the Society shall furnish to the Registrar the full text and necessary illustrations at least a week before the meeting of the Committee of Science at which he desires publication to be considered. Meetings of the Committee of Science are usually held on the second Tuesday of each month from November to June inclusive.
>
> "A paper may be published either in the Scientific Proceedings or the Economic Proceedings of the Royal Dublin Society, as directed by the Committee of Science. The copyright of papers so published shall be the property of the Society.
>
> "Fifty copies of each paper shall be supplied free of cost to the Author or Authors.
>
> "Additional copies may be obtained at Cost Price if applied for by the Author when returning the proofs."

Authors are requested to apply to the Registrar for further information concerning the preparation of papers and illustrations for press.

Authors of biological papers involving extensive numerical observations, are requested to consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from the British Association, Burlington House, W. 1, price 6d. Arrangements have been made whereby original data, too extensive for complete publication, may be deposited at the British Museum (Natural History), or with the Royal Society of Edinburgh, and so be made available for future workers. Authors desirous of depositing data, whether zoological or botanical, in this manner, should communicate with either the Keeper of Zoology, Natural History Museum, S.W. 7, or the General Secretary, The Royal Society of Edinburgh, 22 George Street, Edinburgh, enclosing full bibliographic reference to the publication in which the results are summarised.

No. 22.

INVESTIGATIONS ON THE CONTROL OF SEEDLING DISEASES OF SUGAR BEET.

By WILLIAM HUGHES, B.Agr.Sc., M.Sc.,
Albert Agricultural College, Glasnevin, Dublin.

[Communicated by Dr. P. A. Murphy.]

[Read March 26. Published separately April 10, 1935.]

The sugar beet crop in the early stages of its growth is particularly liable to destructive attacks of parasitic fungi, resulting in a diseased condition of the seedlings which is commonly known as blackleg. The attack usually occurs at or below the level of the soil, at which point the stem of the plant becomes discoloured. The disease progresses downwards into the roots, and eventually the affected portion becomes black and shrivelled. Finally, the seedling wilts and dies.

The fungus *Phoma Betae* (Oud.) Frank is held to be chiefly responsible for the disease, while soil-inhabiting organisms such as *Pythium de Baryanum* Hesse, *Aphanomyces laevis* de Bary, and *Rhizoctonia Solani* Kühn, and other fungi may be associated to a lesser extent. *Phoma Betae* is carried with the seed,[1] and may occur in very large amounts in ordinary commercial clusters. In recent continental investigations Schmidt (3) found that 40 to 100 per cent. of the seed was commonly infected. Verplancke (4), who examined twenty commercial Belgian samples, found that all were infected, although six of them showed no external trace of disease. The beet seed used in Ireland is grown on the continent of Europe, and it is probable, in view of these results, that considerable quantities of infected seed are grown every year, while the moist conditions which usually prevail here in the late spring provide a suitable environment for the development of blackleg. It is therefore *a priori* probable that considerable benefit is likely to be derived from treatment of the seed with suitable chemicals.

The present investigation was undertaken in the first instance at the request of the Department of Agriculture and the Irish Sugar Co., in order to determine whether the treatment applied to the seed in bulk by the continental producers

[1] Throughout this paper the term "seed" is used in the popular sense, and refers to the fruit clusters of commercial samples.

was effective and necessary. An untreated sample of the same seed was supplied by the Sugar Co., and this was dressed at home with various disinfectants (including the material used in the bulk treatment), while a portion was left untreated. The experiment, therefore, permitted of the following comparisons :—

1. Bulk treatment abroad before shipment, compared with treatment in small lots at home before sowing.
2. Comparisons of various disinfectants, dusts and liquid, applied before sowing.
3. Comparison of treatment with no treatment.

The experiments were carried out at five centres, but at Glasnevin only was it possible to plan the work in such a manner as to render the results capable of accurate interpretation. At the other centres uncontrolled factors obscured the effect of seed treatment, and the results from them are not included.

The author desires to acknowledge his indebtedness to the Irish Sugar Co. for the supply of beet seed, and to the Department of Agriculture for their co-operation. The investigation was under the direction of Dr. P. A. Murphy, Professor of Plant Pathology, Albert Agricultural College, Glasnevin.

Field Experiment at Glasnevin in 1934.

Disinfectants.—The materials used at Glasnevin included seven proprietary dusts and one liquid seed disinfectant, all applied from one to four days before sowing. These were compared with a dust treatment applied in bulk by the continental seed producers. The liquid treatment (Germisan) was used in a 0·25 per cent. solution, the seed being steeped in this for two hours and then spread out to dry preparatory to sowing. The dusts used included Agrosan G, Ceresan (U.T. 1875 A), Ceresan (old), Granosan, Hortosan A, Hortosan B, and Sch. 1124 A, and they were all used at 1·5 ounces per bushel or 9·5 ounces per cwt. (1 part powder to 189 parts of seed). Ceresan (old) was stated to have been used by the producers for their treatment in bulk. The seed used was of the variety Kühn P, and was supplied by the Irish Sugar Co.

Examination of seed clusters for infection.—Five lots, of 100 seeds each, from the untreated sample were steeped in water, and examined directly under the lower power of the microscope for the presence of pycnidia of *Phoma Betae,* with the following results :—

Number of seeds examined	100	100	100	100	100
Number of seeds bearing pycnidia	12	9	18	13	8

The average number of clusters infected was 12 per cent. This method of examination is not entirely reliable quantitatively, but it shows that a high percentage of infection was present, which, given suitable conditions, would

be capable of causing a serious outbreak of disease and a considerable reduction of "plant." The seriousness of the infection is better appreciated if expressed in terms of potential sources of disease in the field experiment outlined below. It is found on the above basis that there would be 75 infected clusters per square perch, which means that 36 per cent. of the dibble holes would be likely to contain a possible source of the disease.

Germination of the seed.—Samples of the treated and untreated seed were examined at the Seed Testing Station to determine the effect of treatment on germination. The results are given in the following table, in which the treatments are listed in the order of excellence shown in the field:—

TABLE 1.—*Germination of Seed as Determined in Seed Testing Station.*

Treatment.	Germination of clusters.	Seedlings per cent. After 7 days.	Seedlings per cent. After 14 days.
1. Germisan (liquid) ...	95	169	177
2. Ceresan (U.T. 1875 A) ...	90	164	177
3. Granosan	93	170	178
4. Ceresan (old)	94	174	183
5. Ceresan (old) applied by producers	90	144	156
6. Hortosan B	91	163	172
7. Sch. 1124 A	91	159	167
8. Agrosan G.	92	165	172
9. Hortosan A	94	169	175
10. Untreated	92	168	175

It was not possible to examine these figures statistically, but there is no evidence of a stimulating effect on germination, as this, if present, would be likely to influence the number of seedlings produced in 7 days. As will be seen this number is comparatively uniform throughout. The production of seedlings by the untreated seed compares favourably with that of the treatments which gave the best results in the field experiment (see Table 2). It is significant, however, that a marked decrease occurred in the seedlings derived from the seed treated by the producers, and this would suggest that the lapse of time between treatment and sowing was responsible for some injury to the seed in this case.

Plan of field experiment.—The experimental plot had an area of approximately one-half acre, and comprised 50 drills 86 yards long. These were divided into 5 blocks of 10 drills each, within which the nine treatments and one control were distributed at random. There were therefore five replications of each treatment and control. The seed was sown by hand in dibble holes 9 inches apart, three seeds being placed in each hole with the greatest possible care. The arrangement of the experiment rendered the results capable of statistical analysis for the estimation of experimental error.

Effect of treatments on emergence of seedlings.—Following sowing on the first, second, and third of May, 1934, during dry warm weather, the seedlings began to appear on May 13th, but there were no consistent differences in the time of emergence in the various plots, although considerable variation was evident. The absence of a stimulating effect on germination in the field is in agreement with the results of the seed test already referred to.

At the time of singling (June 15th) a count was made of the seedlings on one square perch in each plot, the results of which are given in the following table:—

TABLE 2.—*Number of seedlings present in the field at singling time (June 15th).*

Treatment	Plots. 1	2	3	4	5	Treatment Totals (5 sq. per.)	Increase over control	Per cent. increase.
1. Germisan (liquid) ...	681	802	735	762	710	3,690	799	27·6
2. Ceresan (U.T. 1875 A)	740	650	744	738	816	3,688	797	27·6
3. Granosan	658	817	664	807	684	3,630	739	25·6
4. Ceresan (old) ...	777	668	678	731	666	3,520	629	21·7
5. Ceresan (old) applied by producers ...	730	566	551	764	676	3,287	396	13·7
6. Hortosan B	609	452	577	704	728	3,070	179	6·2
7. Sch. 1124 A ...	563	579	700	607	576	3,025	134	4·6
8. Agrosan G	638	631	605	468	578	2,920	29	1·0
9. Hortosan A	626	700	550	492	545	2,913	22	0·8
10. Untreated	587	639	602	480	583	2,891	—	—

The treatment totals differ considerably, and some of them show large increases over the control, suggesting that the disinfectants used in these cases exercised a strong influence on plant establishment. To secure exact data for comparing the effect of treatments, the above figures were analysed statistically by Wishart's (5) application of Fisher's analysis of variance method, with the following results:—

Analysis of variance due to:—	Degrees of Freedom	Sums of Squares.	Mean Squares.
Blocks	4	2,379·48	594·87
Treatments	9	207,338·48	23,037·61
Error	36	211,864·92	5,885·14

$Z = 0·6823$. Standard error = 171·54.

It is clear from this, that the treatment mean square (23,037·61) is greatly in excess of the error mean square (5,885·14). This difference, as determined by the Z test (2), is found to be significant, and the results concerning the individual disinfectants may be further examined to determine what differences are real. The standard error of a single plot is the square root of the error mean square (5,885·14), and that of each treatment (which is a total of 5 plots) is $\sqrt{(5885·14 \times 5)}$. The standard error for the group of 5 plots is therefore

171·54. To be significant, the differences between any two treatment totals should exceed three times the standard error, that is 515.

Comparison of the effectiveness of individual treatments.—Comparing treatment with no treatment, the increase in plant from the first four disinfectants over the untreated control was greater than 3 times the standard error, and the figures may, therefore, be regarded as significant. In the case of the remaining five disinfectants the increases over the control were not significant. The four significantly effective treatments were Germisan, Ceresan (U.T. 1875 A), Granosan, and Ceresan (old). The five ineffective materials, namely the seed producer's bulk treatment (Ceresan—old), Hortosan B, Sch. 1124 A, Agrosan G, and Hortosan A, form, along with the control, a group containing no significant differences.

The treatment with Ceresan (old) just before sowing was clearly superior to the bulk treatment on the Continent with the same material. It would probably be reasonable to expect that the latter might prove somewhat inferior on account of subsequent loss of dust, possibly less even application, and a certain injury due to too long contact with the seed, as the Seed Testing Station figures suggest. In the light of these results the conclusion has been reached in Ireland that it is inadvisable to purchase seed which has already been treated, in view of the added cost and inferior results, particularly as large stocks are sometimes held over from one year to another. Woodward and Dillon Weston (6) have shown in England that beet seed which was supplied as having been treated (presumably in bulk) produced a high percentage of diseased seedlings.

The connection between disease and seedling count.—In the present experiment, the number of seedlings which developed blackleg among the plants that appeared overground was negligible. Although no direct attempt was made to determine to what extent the reduced plant resulting from no treatment or ineffective treatment was due to the failure of attacked seedlings to emerge, since the scale of the experiment precluded such detail, an examination of the results shows that there was a direct connection between the two occurrences. The examination at the Seed Testing Station showed that there was no direct stimulation to germination, but this could not take into account seedlings which might have been weakened by disease, and succumbed afterwards. In the field, however, the ultimate criterion of effective germination was the number of surviving healthy seedlings, and inhibitory factors operating subsequent to germination became operative there, which did not affect the germination tests. For example, in this experiment the number of seedlings which might have been produced on five square perches from untreated seed was 5,460, the estimate being based on the Seed Testing Station count of seedlings produced in 14 days. The actual number of seedlings at the time of singling was 2,881, or 53 per cent. of the possible total. There is thus a difference of 47 per cent. to be accounted for, and considering the largely increased emergence due to

certain disinfectants, it is clear that a high percentage of the failures was caused by disease originating from the seed, and operating principally by killing the seedlings below ground.

In a greenhouse experiment carried out by Woodward and Dillon Weston (6) 91 per cent. of the diseased seedlings remained below soil level. This result was secured under conditions which, presumably, were very favourable for infection, and field conditions might not be strictly comparable. The fact is significant, however, that a large percentage of the seedlings lacked the vigour to grow overground owing to the action of disease, and the beneficial effects of certain disinfectants in both investigations is, therefore, to be explained by the elimination or reduction of this injury.

Later development of the crop.—In the development of the crop after singling, the disease had little or no effect, as is evident from a comparison of Tables 2 and 3. It will be seen that there is a general agreement between the final number of roots and the number of seedlings, for those treatments which, as a group, gave the best results at singling time also gave the highest plant population in the final crop. The differences in the final number of plants, however, were not so great. For example, Germisan was responsible for an increase of 5·3 per cent. in the number of mature roots, but it increased the seedlings by 27·6 per cent. It is clear that a sufficient number of vigorous seedlings was available at singling time, even in the untreated seed plots, to ensure a comparatively good plant, and that disease had no serious influence afterwards.

TABLE 3.—*Final number of roots per acre.*

Treatment.	No. of roots per acre in final crop.	Per cent. of total possible number.
1. Germisan (liquid)	31,264	94·2
2. Ceresan (U.T. 1875 A)	31,200	94·0
3. Granosan	31,328	94·4
4. Ceresan (old)	31,008	93·4
5. Ceresan (old) applied by producers ...	29,856	90·0
6. Hortosan B	29,920	90·1
7. Sch. 1124 A	29,728	89·6
8. Agrosan G	30,016	90·4
9. Hortosan A	29,664	89·4
10. Untreated	29,504	88·9

Yield.—An estimate was made of the yield of roots on the plots on which untreated and Germisan-treated seed was grown, as these represented the lowest and highest plant, respectively, in the seedling stage. The result was as follows :—

	Tons per ac.	Av. wt. of root in lbs.
Germisan	17·0	1·2
Untreated	16·0	1·2

It is clear, in view of the high yield on the control plot, that the disease did not seriously affect the crop after singling. The estimated average root-interval in the controls was 10·1 inches, while that in the Germisan plots was 9·5 inches, and the difference in yield is attributable to this. In practice the root-interval of the controls would be regarded as very good, and is probably much closer than in beet-growing areas generally. Dowling (1) in emphasising the necessity of a root-interval of 10 inches for profitable beet-growing, states that the average plant in English districts is about half the optimum figure. The importance of this consideration will be realised when it is stated that on 21-inch drills with a root-interval of 9 inches, the number of plants per acre would be 33,188. If, through failures, the average root-interval were increased to 12 inches the number of plants would be reduced to 24,891, that is a reduction of 8,297 roots. Irregular spacing of this type can easily pass unnoticed, although frequently of greater dimensions, and is of common occurrence. It is probably responsible for a greater annual loss than obvious total failures.

As it turned out, none of the treatments, even the most successful, produced a significantly bigger crop than the untreated seed under the conditions of the experiment. It is likely, however, that had circumstances been more suitable for the development of blackleg, the loss of seedlings in the pre-singling stage would have been reflected in the final crop. It is well known that under certain conditions of weather and soil, there may be a partial or even a complete failure of the crop, due to the seedlings not coming up, or failing to survive afterwards. An effective seed treatment reduces this risk, and in some cases it is likely to make all the difference between a full crop and a poor one. According to present information, therefore, treatment of the seed is to be looked upon as a form of insurance, and should be practised accordingly. This conclusion supports that already reached in other countries where sugar beet growing has been longer established.

SUMMARY.

1. A trial was made of the effectiveness of sugar beet seed treatment in bulk, as carried out by the continental producers, in comparison with treatment with various disinfectants in small lots just before sowing, and with no treatment.

2. The seed used, which was Kühn P, showed 12 per cent. of the clusters visibly affected with *Phoma Betae.*

3. As determined in the Seed Testing Station, none of the treatments stimulated or increased germination, but the bulk treatment notably reduced it, probably due to too long contact between the seed and disinfectant.

4. In a randomized field experiment, the results of which were statistically analysed, the following materials gave a significant increase in the number of

seedlings over the control—Germisan and Ceresan (U.T. 1875 A) (27·6 per cent.), Granosan (25·6 per cent.) and Ceresan (old) 21·7 per cent.

5. The remaining treatments, which included the seed producers' treatment in bulk, were not significantly better than the control.

6. Disinfection of the seed in small lots before sowing gave better results than treatment in bulk by the seed producers.

7. The increase in the number of seedlings is attributed to the effect of the disinfectants on *Phoma Betae,* mainly in preventing attacks on the seedlings below ground.

8. Subsequent to singling, the disease had no appreciable effect on the final number of plants or on the yield. Nevertheless, seed treatment is recommended as a necessary insurance.

Literature Cited.

1. Dowling, R. N.—Factors in sugar beet profits. Farmer and Stockbreeder Year Book and Livestock Journ. Annual, 162–164, 1934.

2. Fisher, R. A.—Statistical methods for research workers. 4th edn., 1932.

3. Schmidt, E. W.—Ueber den Wurzelbrand der Rüben. Eine Kritische Betrachtung. Deutsche Zuckerind., **59**, 520–521, 1934. Ref. Rev. Appl. Myc., **13**, 742.

4. Verplancke, G.—Étude comparative de glomérules de Betteraves. La Sucrerie Belge, **53**, 361–377; 385–392, 1934. Ref. Rev. Appl., Myc., **13**, 742.

5. Wishart, J.—Methods of field experimentation and the statistical analysis of the results. Rothamsted Conferences, **13**, 13–21, 1931.

6. Woodward, R. C., and Dillon Weston, W. A. R.—Treatment of sugar beet "seeds" to prevent seedling diseases. Ann. Appl. Biol., **16**, 542–566, 1929.

No. 23.

ON THE CHARACTERISTICS OF *BACTERIUM VIOLACEUM* (SCHRÖTER) AND SOME ALLIED SPECIES OF VIOLET BACTERIA.

By GEORGE CRUESS-CALLAGHAN, M.Sc.,
AND
M. J. GORMAN, A.R.C.Sc.I.

(Plate 6.)

[Read June 27, 1933. Published separately May 21, 1935.]

Violet bacteria are widely distributed in natural waters, and consequently are often met with in laboratories in which the bacteriological examination of samples of water is part of the routine. As these organisms are adapted to rather low temperatures (few of them make any visible growth at 37° C.) the violet colonies usually appear on plates incubated at 20° C. or thereabouts. During the past fifty years or so several studies of violet bacteria isolated from water have been made in Europe and America, and descriptions of many species, sub-species, and strains, are to be found in publications dealing with systematic bacteriology. The first published account seems to be traceable to Schröter (11), who in 1872 introduced the name *Bacteridium violaceum,* and the next to Bergonzini (3), whose detailed description of *Cromobacterium violaceum* appeared in 1881. These two names are now regarded as synonyms. Four years later Zopf (13) gave the name *Bacterium janthinum* to an organism which by some authors is regarded as identical with, and by others as distinct from, the foregoing species. *Bacillus membranaceus amethystinus* Eisenberg appears in 1891, and in Flügge's (5) systematic work published in 1896 descriptions of six species and sub-species under the generic name *Bacillus* are included. Migula (8) placed these violet bacteria, which he found to have polar flagella, in his genus *Pseudomonas*; others of them he placed in *Bacterium,* giving details of six species in all.

Bampton (1) made extensive cultural and serological studies of twenty-two cultures collected from various laboratories, and found that while many of the cultures resembled one another very closely no two of them agreed exactly in all characters. He came to the conclusion, however, that all his cultures could be divided into two specific groups, viz., *violaceus* and *membranaceus amethystinus.*

Lehmann and Neumann (7) in the latest edition of their well-known systematic work recognise only two species—*Bacterium violaceum* (J. Schröter) L. and N., and *Bacillus membranaceus amethystinus* Eisenberg, holding that *Bact. janthinum* Zopf, *B. lividus* Flügge and Proskauer, and other forms claimed as species by various authors cannot be separated from *Bact. violaceum.*

Buchanan (4) revived *Chromobacterium* (originally used by Bergonzini as *Cromobacterium*) as a generic name for bacteria producing a violet pigment soluble in alcohol but not in chloroform. The term was adopted by the Committee of the Society of American Bacteriologists (1920), and in the latest edition of Bergey's Manual (2) ten species of Chromobacterium are described, the type being *Chromobacterium violaceum* (Schröter) Bergonzini. Six of the species, viz., *Chr. violaceum, Chr. janthinum,* three *membranaceus amethystinus* forms, and *Chr. lividum,* seem to form a natural group distinct as regards nature of pigment and other features from the remaining four species. Finally Grimes claims as new species two forms which he has recently described under the names *Chr. cohaerens* and *Chr. hibernicum.*

The present study may be said to have had its origin in our failure to identify satisfactorily a number of violet organisms met with in samples of water. These specimens, while clearly agreeing in many of their characters with *Bact. violaceum* as described in current systematic literature, yet in other respects seemed sufficiently divergent to suggest the possibility that there might be among them representatives of new species. It was found, for example, that the freshly-isolated forms freely reduced nitrate to nitrite,[1] a property which is stated explicitly to be lacking in all the species of violet bacteria as described in Bergey's Manual. Furthermore, the flagella appeared to be unipolar, whereas in the type species, at all events as described in the works of Bergey and of Lehmann and Neumann, the flagella are given as peritrichous. Some cultures were then obtained from London and elsewhere, and these too with few exceptions appeared to be organisms bearing a single polar flagellum, and having the ability to reduce nitrate. These results led to the decision to secure cultures of violet bacteria from as many sources as possible for the purpose of making a comparative study, and with much courtesy a number of institutions in Europe and America kindly sent us cultures of any violet bacteria which they happened to have. Eighteen cultures were received in this way, and these, together with six freshly isolated, were studied as far as possible concurrently, and their morphological, cultural, and physiological characters recorded. For convenience the reference numbers of the cultures as used in the text, and an indication of their sources are given here:—

Reference numbers.	*Culture received from.*
16, 17, 18, 19.	Delft (Dr. Kluyver).
2, 13, 14, 15.	London (Lister Institute).
22.	Cork (Dr. Grimes).
23.	Chicago (Amer. Type Cult. Coll.).
27.	Hamburg (Dr. Neumann).

[1] It was on account of their ability to reduce nitrate to nitrite that Grimes (6) was led to regard as new species the violet bacteria which he described. Flügge (5) recorded nitrate reduction for *B. violaceus Laurentius* and *B. violaceus Berolinensis,* both, according to Lehmann and Neumann, synonyms of *Bact. violaceum* Schröter.

Reference numbers.	*Culture received from.*
28.	Berlin (Dr. Bürger).
29.	Munich (Dr. Kiskalt).
30, 31, 32, 33, 34.	Breslau (Dr. Prausnitz).
1, 20, 21, 24, 26, 35.	Dublin (recently isolated).

The materials and methods employed call for little comment. The nutrient agar, gelatine, and other media, were prepared according to ordinary laboratory formulae. Sugar broths and milk were tested for changes in reaction by means of the quinhydrone electrode apparatus. For the indole tests tryptophane broth was used. The technique of Plimmer and Paine (9) was adopted for flagella staining. All cultural characters and physiological reactions were recorded from cultures incubated at 20° C. When the complete results were tabulated it was found that, though all the cultures agreed in several important features and were clearly members of a single natural group, nevertheless they varied quite widely in details. All produced in varying amounts the characteristic violet pigment soluble in alcohol but not in water. On three-day agar slopes at 20° C. the organisms appeared as stoutish or slender rods with rounded ends, occurring singly or occasionally in short chains, and varying in dimensions within the limits 0·4–0·8μ by 1·0–6·0μ. None formed endospores. All were Gram negative and motile by means of a single polar flagellum, or less frequently by means of a few peritrichous flagella. As would be expected, it was with respect to the physiological reactions that the greatest variations occurred among the cultures. The optimum temperature for all appeared to be 20–25° C.; a few grew at 37° C., and most of them at 2–4° C. None produced gas in any of the sugar broths. All but two of the cultures formed acid in glucose broth, nearly half of them gave acid in maltose, and two gave faint traces of acid in saccharose, while all the lactose cultures became distinctly alkaline. In litmus milk none formed curd. Several decomposed the casein, and several decolourised the indicator. None of the cultures formed indole in tryptophane broth. All but a few, in which the reaction was very slight or absent, freshly reduced nitrate to nitrite. On potato growth was variable, being in most cases scanty.

Several authors have recorded their observations regarding the number and arrangement of the flagella in the violet bacteria. Migula (*l.c.*, p. 940) it will be recalled maintained that *violaceum* has a single polar flagellum (hence the name *Pseudomonas violacea*). He had examined thirty cultures before coming to this conclusion, and suggests that Lehmann and Neumann, who in all editions give the flagella of *Bacterium violaceum* as 3-4 peritrichic, 1-2 polar, are dealing with a different species. The latter authors, however, point out that in poor preparations all but one of the flagella may fall off, with the result that the flagellation, which is in fact peritrichic, appears to be monotrichic. Bampton (*l.c.*) simply mentions that the organisms are motile by means of 3-4 peritrichic flagella and 1-2 polar, but does not refer to any method of

staining. Wooley (12), describing *Chromobacterium violaceum Manilae*, states that "each organism possesses a polar flagellum. In rare instances two flagella may be distinguished springing from the same part of the bacillus." Bergey (*l.c.*) distinguishes peritrichous, monotrichous, and non-motile forms. In view of these divergent conclusions a considerable amount of attention was given in the present study to the matter of flagella staining, and briefly stated three kinds of result as illustrated in Plate 6 were obtained, viz.—

(i) Well stained peritrichic (about 5–7) flagella (figs. 1 and 2). Found in cultures 13, 23.

(ii) Preparations showing a single polar flagellum only, and bearing no indications that other flagella had been present and had been shed (figs. 5 and 6). Observed in cultures 1, 2, 20, 24, 17, 28, 31, 31, 32, 33, 34.

(iii) Preparations in which the organisms showed a single polar flagellum, but a few individuals were found, usually after prolonged searching, in which more than one flagellum was present (figs. 3 and 4). Seen in cultures 16, 19, 26, 27.

This result is not entirely satisfactory, and the matter is under further investigation. In the meantime there seems to us no doubt that some forms of violet bacteria have peritrichic flagella and others unipolar, just as is admittedly the case in closely related non-chromogenic groups.

It was only when the results from all the cultures had been tabulated that any really satisfactory attempt could be made to reach a decision regarding the systematic position of each. No single one of them was found to correspond in detail with any of the species described in the literature, but at the same time some doubt was felt regarding the systematic importance of the variations, and in the end the conclusion was reached that formation of new species based on such variations would not be justified. Careful comparison of our results with the descriptions by the various authors enabled us ultimately to arrange the cultures or strains in three specific groups corresponding to *Bact. violaceum* (Schröter) (Nos. 1, 2, 17, 20, 24, 28, 30, 31, 32, 33, 34); *Bact. janthinum* (Zopf) (Nos. 13, 14, 15, 23); and *Bact. membranaceum amethystinum* (Nos. 16, 18, 19, 21, 22, 26, 27, 29, 35, 36).

In each of these groups the only strain of which the full detailed description need be given is that one which approaches nearest to the type on which the species was originally founded. For the others it will be sufficient merely to refer to those characters in which they have been found departing from the type. The strains selected for detailed description are therefore: No. 2, representing *Bacterium violaceum* (Schröter); No. 13, representing *Bacterium janthinum* (Zopf); and No. 16, *Bact. membranaceum amethystinum* (Eisenberg).

STRAIN NO. 2, REPRESENTING *Bacterium violaceum* (Schröter).

Morphology.—Rods 1·8–3·5μ long, 0·4–0·6μ wide, occurring singly, in pairs, and short chains. Motile by means of a single polar flagellum. Gram negative.

Cultural and Physiological Characters at 20° C.

Gelatine colonies.—3 days: surface colonies circular, violet, 3 mm., densely "woolly" at centre, less so towards margin, which is finely ciliate: saucer-shaped liquefaction; submerged colonies smaller, creamy.

Gelatine stab.—24 hours: filiform growth along stab; surface violet; 5 days: liquefaction well advanced, infundibuliform; liquefaction almost complete in 10 days; growth in liquid whitish.

Agar colonies.—2 days: surface colonies, 3 mm., violet, with light-violet ciliate margin; 4 days: up to 7 mm. in diameter, centre deep violet, outer zone lighter in colour with ciliate margin. *Sub-surface colonies.*—4 days: under 1 mm., violet.

Agar slant.—Violet streak, smooth, moist, shiny, with pale irregularly undulate margins; edges ciliate; very slowly spreading.

Broth.—24 hours: turbid; 3–5 days: greyish-violet pellicle, with numerous violet spots; violet sediment; 10 days: liquid faintly violet.

Litmus milk.—4 days: no apparent change; 6 days: slight violet ring; 2 weeks: viscous violet surface growth, deep-violet ring; slight peptonisation, pH changed from 6·9 to 7·2.

Potato.—10 days: growth scanty or absent.

Glucose broth.—2 days: cloudy, distinctly acid; 10 days: no pellicle, no sediment; 2 weeks: pH changed from 7·1 to 5·3.

Maltose broth.—2 days: cloudy, distinctly acid, fragile violet pellicle; 5 days: greyish-violet sediment; 2 weeks: pH changed from 7·1 to 5·8.

Lactose broth.—2 days: cloudy, no acid, fragile, greyish-violet pellicle; 5 days: violet pellicle, violet sediment; 2 weeks: pH changed from 7·1 to 7·5.

Sucrose broth.—As lactose broth.

Indole.—Not formed.

Nitrates.—Actively reduced to nitrites.

Temperature.—Optimum, 20° C.; no growth at 37° C.; growth slow at 25° C.; slight growth at 2°–4° C.

Nos. 1, 20, 24, 28, 32, 33 all resemble No. 2 very closely in cultural and physiological details, differing only slightly in such characters as texture of colony on agar, rate of liquefaction of gelatine, and of peptonisation of milk, and rate of growth at temperatures above 25° C. and below 20° C. Nos. 30 and 31 are somewhat larger than the type (2·0–5·0μ long by 0·5–1·0μ wide) and do not produce acid in maltose. No. 32 is indistinguishable from No. 33,

and No. 17 from No. 34. These latter two differ from No. 2 only in the growth on agar showing a tendency to spread.

STRAIN No. 13, REPRESENTING *Bacterium janthinum* (Zopf).

Morphology.—Rods 1·0–3·0μ long, 0·2–0·3μ wide, occurring singly, occasionally in pairs and short chains. Motile by means of 5-7 peritrichic flagella. Gram negative.

Cultural and Physiological Characters at 20° C.

Gelatine colonies.—3 days: surface colonies greyish up to 8 mm. in diameter, saucer-shaped liquefied area; sub-surface colonies greyish or creamy, 1 mm.

Gelatine stab.—24 hours: grey filiform growth along stab; surface growth violet; 5 days: liquefaction well advanced; infundibuliform, heavy white flocculent growth in liquid; 10 days: gelatine one-quarter liquefied.

Agar colonies.—4 days: surface colonies, 2·5 mm., creamy centre, violet margin, smooth, moist, shiny, edges even, entire; sub-surface colonies, 0·3 mm., colourless; still colourless after 2 weeks.

Agar slant.—Deep violet growth confined to streak; surface smooth, moist, shiny, edge even, margin entire.

Nutrient broth.—2 days: turbidity; 5 days: slight violet sediment; 10 days: very slight violet ring, increased violet and greyish sediment.

Litmus milk.—4 days: no apparent change; 6 days: marked peptonisation, which is almost complete in two weeks; slight violet ring; litmus decolourised from below upwards.

Potato.—4 days: moderate growth; dark violet streak; smooth, shiny, potato darkened.

Glucose broth.—2 days: turbidity; 5 days: violet ring, greyish sediment; 2 weeks: reaction changed from pH 7·1 to pH 5·3.

Maltose broth.—2 days: turbidity; 5 days: violet ring, slight grey sediment; 2 weeks: reaction changed from pH 7·1 to pH 7·6 (or over).

Lactose broth.—As maltose broth.

Saccharose broth.—As maltose broth.

Indole.—Not formed.

Nitrate.—Slight or no reduction to nitrite.

Temperature.—Optimum, 20°–25° C.; good growth at 37° C.; no growth at 2°–4° C.

Strains Nos. 14, 15, and 23 are indistinguishable from No. 13.

STRAIN No. 16, REPRESENTING *Bacterium violaceum amethystinum* (Eisenberg).

Morphology.—Rods 1·5–3·0μ long, 0·3–0·5μ wide, occurring singly, in pairs, and occasionally in short chains. Motile by means of a single polar flagellum; peritrichic flagella were seen in a few cases. Gram negative.

Cultural and Physiological Characters at 20° C.

Gelatine colonies.—4 days: violet centre, outer zone colourless, hazy, saucer-shaped; sub-surface colonies all colourless; little or no liquefaction.

Gelatine stab.—24 hours: grey filiform growth along stab, slight violet surface growth. In 2 weeks the surface growth develops into a heavy violet-black pellicle; ¼-inch stratiform liquefaction; slight hazy growth along stab.

Agar colonies.—surface; 2 days: up to 1·25 mm. diam., smoky or bluish; 4 days: deep violet, 2 mm., surface rugose, edges undulate; 4 weeks: slightly elevated ridge around the undulate margin. Sub-surface colonies remain small and colourless.

Agar slant.—24 hours: smoky streaks with specks of violet; 4 days: deep violet streak, surface slightly raised, uneven, glistening; edge irregularly undulate; coherent and easily removed.

Nutrient broth.—2 days: turbidity; fragile, violet pellicle; 3 days: violet pellicle and granular violet growth near top; 5 days: deep violet flecks throughout broth, violet sediment. Sediment increases and broth becomes violet.

Litmus milk.—6 days: slight violet surface growth; 4 weeks: faint signs of peptonisation; milk deep blue.

Potato.—2 weeks: deep violet, rugose, spreading growth: potato slightly browned.

Glucose broth.—24 hours: turbidity; 6 days: pale violet pellicle, no acid; 2 weeks: pH changed from 7·1 to 6·7.

Maltose broth.—24 hours: turbidity; 6 days: heavy, deep violet wrinkled pellicle, violet sediment; 2 weeks: pH changed from 7·1 to 7·6.

Lactose broth.—As maltose.

Saccharose broth.—As maltose.

Indole.—Not formed.

Nitrates.—Actively reduced to nitrites.

Temperature.—Optimum, 20°–25° C.; no growth at 37° C.; good growth in 7 days at 2–4° C.

Nos. 18, 19, 27 agree almost exactly in detail with No. 16. Nos. 21, 22, 26 resemble one another very closely; none of them give acid in glucose, maltose, or sucrose broths. Nos. 29 and 35 also approach the type closely, but give traces of acid in maltose and sucrose as well as in glucose.

Synopsis of contrasting Characters of the three species.

Bacterium violaceum (Schröter).

Flagella polar.
Acid in glucose and maltose; no acid in sucrose.
Nitrates reduced.
Growth on potato scanty or absent.
No growth at 37° C.; growth at 2–4° C.

Bacterium janthinum (Zopf).

Flagella peritrichic.
Acid in glucose; no acid in maltose or sucrose.
Nitrates not (or slightly) reduced.
Growth on potato moderate.
Good growth at 37° C.; no growth at 2–4° C.

Bacterium membranaceum amethystinum (Eisenberg).

Flagella peritrichic (?)
Slight or no acid in glucose, maltose, sucrose.
Nitrates reduced.
Growth on potato abundant.
No growth at 37° C.; good growth at 2–4° C.

Summary and Acknowledgments.

Twenty-four strains of violet bacteria, six of which had recently been isolated from water and the remainder obtained from laboratories and collections, were studied morphologically, culturally, and physiologically.

Practically all the cultures differed in some degree from one another, but the conclusion was reached that they represented three species, viz.: *Bact. violaceum* (Schröter), *Bact. membranaceum amethystinum* (Eisenberg), and *Bact. janthinum* (Zopf).

Both polar and peritrichous forms of flagellation were found, the polar being much the more frequent. All but three of the strains reduced nitrate to nitrite.

In addition to those whose names have already been mentioned as having supplied cultures and to whom we wish again to express our indebtedness, we desire to thank Miss M. Rhodes, Assistant Curator, Lister Institute, London, for much generous help; also Dr. G. van B. Gilmour for cultures contributed by him and included among those referred to as recently isolated.

EXPLANATION OF PLATE.

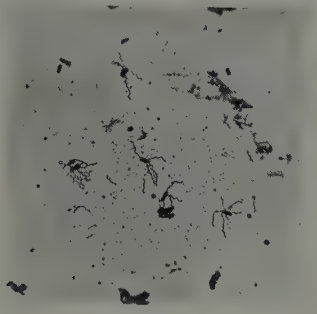
(All magnifications approximately × 1,000.)

FIG. 1.—Peritrichic flagella in strain No. **23** (Bact. janthinum).
FIG. 2.—Peritrichic flagella in strain No. **13** (Bact. janthinum).
FIG. 3.—Polar and peritrichic flagella in strain No. **19** (Bact. membranaceum amethystinum).
FIG. 4.—Polar and peritrichic flagella in strain No. **26** (Bact. membranaceum amethystinum).
FIG. 5.—Polar flagella in strain No. **34** (Bact. violaceum).
FIG. 6.—Polar flagella in strain No. **18** (Bact. membranaceum amethystinum).
FIG. 7.—Polar flagella in strain No. **2** (Bact. violaceum).
FIG. 8.—Peritrichic flagella in strain No. **29** (Bact. membranaceum amethystinum).

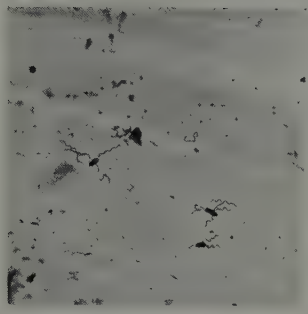
REFERENCES.

1. BAMPTON, J. H.—Ueber Violaceus und Membranaceus amethystinus. Cent. f. Bak. 1 Abt. (orig.), **71**, 129–146. 1913.
2. BERGEY, D. H.—Manual of Determinative Bacteriology. 4th Edn. Baltimore. 1934.
3. BERGONZINI, CURZIO.—Sopra un nuovo Batterio Colorato. Annuario della Societa dei Naturalisti in Modena. Ser. 2, **14**, 149–158. 1881.
4. BUCHANAN, R. E.—Studies in nomenclature and classification of the bacteria. Jour. Bact., **3**, 29–61. 1918.
5. FLÜGGE, C.—Die Microorganismen. Leipzig. 1896.
6. GRIMES, M.—A study of two new species of bacteria belonging to the genus Chromobacterium. Sc. Proc. R. Dub. Soc., **19** (N.S.), 381–384. 1930.
7. LEHMANN, K. B., and NEUMANN, R. O.—Bacteriology. 7th Edn. Trans. Boysen, H. H. et al. New York. 1931.
8. MIGULA, W.—System der Bakterien. Jena. 1897.
9. PLIMMER, H. C., and PAINE, S. G.—A new method for the staining of bacterial flagella. Jour. Path. and Bact., **14**, 286–288. 1921.
10. RAMCHANDANI, J. C.—Saltations in Bacteria. III Bacillus violaceus. Ann. Bot., **44**, 975–989. 1930.
11. SCHRÖTER, J.—Ueber einige durch Bacterien gebildete Pigmente. Cohen's Beitrage Z. Biologie der Pflanzen. Heft 2, **1**, 109–126. 1872.
12. WOOLEY, —.—Pub. No. 15, Bureau Govt. Labs. Manila. 1904.
13. ZOPF, W.—Die Spaltpilze. 3rd edn. 1885.

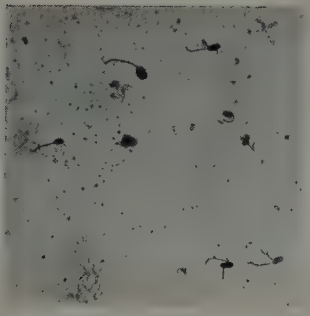
1



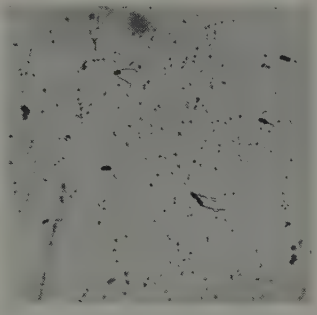
2



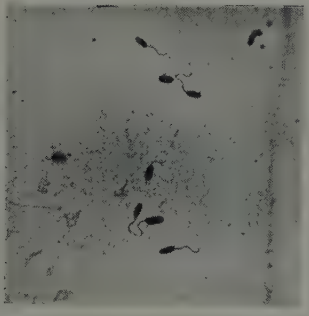
3



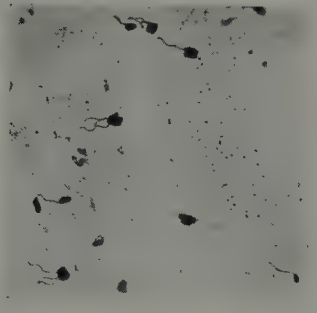
4



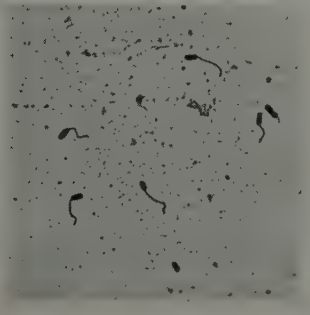
5



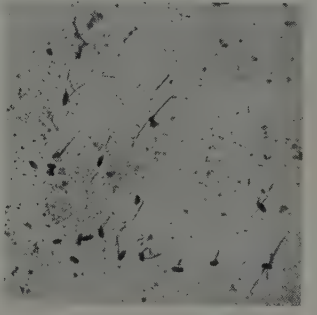
6



7



8



No. 24.

A SIMPLE TITRAMETRIC METHOD FOR THE APPROXIMATE DETERMINATION OF MILK PHOSPHATES.

By G. T. PYNE,

Department of Dairy Chemistry, University College, Cork.

(Communicated by Professor T. J. Nolan.)

[Read April 30. Published separately June 18, 1935.]

In a previous communication to this journal (5) a brief reference was made to an approximate titrametric method for the estimation of soluble phosphate in milk, which it was promised to deal with more fully later. The method has since been somewhat improved, and its present form, here described, includes an extension which makes it possible to determine both types of inorganic phosphate present in milk, viz. the soluble and insoluble (or colloidal), on a single sample to a degree of accuracy of about 5 per cent.—sufficient for many purposes.

It will be best to describe first the titrametric procedure used, and to follow it later by an explanation of the various stages required, and their experimental verification.

Methods.

1. *Soluble Phosphate.*—To 10 c.c. milk are added 5 drops of 0·5 per cent. phenolphthalein, and, with rapid mixing, 1 drop (·03 c.c.) of saturated neutral potassium oxalate. On standing for a minute or two, the milk is titrated with N/10 NaOH to a faint pink, using as a colour standard 10 c.c. of milk containing 1 drop of alcoholic 0·01 per cent. magenta. The use of a standard is essential. An additional 2·0 c.c. N/10 NaOH and then 1 c.c. M/1 $CaCl_2$ are now added, and the mixture allowed to stand for about a minute and back titrated with N/10 acid to the standard tint, stirring constantly. The standard should be diluted to the same degree. The difference between this titration and the 2·0 c.c. excess of NaOH added gives the acidity developed by the formation of tricalcium phosphate from the secondary alkali salt, as represented by the equation

$$2\ K_2HPO_4 + 3\ CaCl_2 = Ca_3(PO_4)_2 + 4\ KCl + 2\ HCl,$$

and is a measure of the soluble phosphate; 1 c.c. N/10 acid = 3·1 mg. P.

2. *Total Phosphate.* — To the re-neutralised milk resulting from the preceding titration is added 1 c.c. saturated neutral potassium oxalate, and the titration with N/10 acid continued, after 5–6 minutes, until the standard neutral tint is again reached. This titration gives the amount of acid required to convert K_3PO_4 (resulting from the action of the oxalate on $Ca_3(PO_4)_2$) into the secondary phosphate according to the reaction—

$$HCl + K_3PO_4 = KCl + K_2HPO_4,$$

and is thus a measure of total inorganic phosphate; 1 c.c. N/10 acid = 3·1 mg. P.

A blank estimation should be made from time to time on 10 c.c. water, using the same quantities of NaOH, $CaCl_2$, and oxalate, in order to allow for the effects of carbonate in the alkali, possible departure of the other reagents from neutrality, etc.

For convenience the first titration will be referred to as the "calcium chloride titration," and the second as the "oxalate titration."

The general principle of the method for phosphates, at least as regards the use of calcium chloride, is familiar, and its applicability for the estimation of pure phosphate solutions of about the same concentration as milk (M/50 to M/100) has been verified also in the present case. There are, however, a few special features in its application to milk which require explanation, or some assumptions, perhaps, which need to be justified, before proceeding to its experimental verification as a whole. These are chiefly the following:—

(*a*) That at the first neutralisation of the milk the soluble phosphate is present almost entirely as the secondary salt.

(*b*) That the addition of alkali and calcium chloride converts this quantitatively into tricalcic phosphate without bringing about at the same time any reaction with other milk constituents which would appreciably displace the pH in either direction.

(*c*) That the final addition of excess oxalate converts into tripotassium phosphate all tricalcium phosphate present, whether formed just previously from the soluble phosphate and calcium chloride, etc., or existing from the beginning in colloidal form (taking this also to be tricalcic), and that no serious development of acidity or alkalinity arises from its action on other milk constituents.

The first two assumptions will now be considered in treating of the soluble phosphate method, leaving the third for discussion later under the total phosphate method.

Soluble Phosphate.

As regards assumption (*a*) the pH of average milk at neutrality to phenolphthalein under ordinary conditions of titration is about 8·7 (6), and thus not far from that for complete formation of secondary phosphate, to which it can be brought still closer by reducing the amount of phenolphthalein used, as is

done here. This fact, while precluding the presence of any appreciable amount of primary phosphate at the end point, does not exclude the formation of some tricalcium phosphate during the titration by interaction between the secondary phosphate and calcium salts always present (7). As it was clearly necessary to prevent this occurring in the present titration, it became essential to reduce in some way the soluble calcium content. Dilution was first tried, but without much success, and was replaced later by the simple device of adding to the sample a small amount of a soluble oxalate. The amount added, viz. 1 drop (·03 c.c.) of sat. potassium oxalate solution per 10 c.c. milk, is regulated to keep well within the limits for soluble calcium in average milk, so as to avoid the danger of bringing into solution any of the insoluble or colloidal phosphate. It does not, therefore, remove *all* soluble calcium, and thus completely exclude some precipitation of tricalcium phosphate during titration, but it reduces this tendency considerably.

As to (*b*)—the possibility of change in the reaction of milk, on addition of calcium chloride, other than that due to soluble phosphate—it is indeed known that a slight development of acidity arises from the action of calcium salts on caseinates. The order of the correction due to this factor, as far as it can be determined by experiments with pure casein solutions, would not appear to exceed about ·05 c.c. N/10 acidity per 10 c.c. milk. It is of course assumed that the casein calcium phosphate complex, in which form casein occurs in milk, will react in much the same way.

From these considerations, then, it would seem that the "calcium chloride titration" for milk should represent in the main the contribution of the soluble phosphate, but may have, however, a tendency to be a little high, owing to the development of a slight degree of acidity through the action of calcium chloride on caseinate, or perhaps a little low owing to a slight premature precipitation of some of the phosphate as the tricalcium salt during the original neutralisation. These tendencies may be expected to counterbalance to some extent, and probably to result in the titration being a fair index of the soluble phosphate alone. How far this is the case, however, is a matter for experiment.

The experimental checking of the technique just described involved the choice of a reliable comparison method for soluble phosphate. Many methods have been used by various workers. These usually involve the preparation of some type of serum, e.g. rennet whey, Chamberland serum, ultra filtrate, dialysate, etc., and the results obtained from these do not always agree too well. A number of the earlier comparisons in the present work (not given) were made against rennet whey, a serum which has frequently been used for this determination, and the phosphate was estimated therein by the oxalate technique which has been shown to give correct values for pure phosphate solutions.

As the agreement was found to be good, the series was continued under more accurate conditions, using skim milk (to obviate the variable and fairly large volume correction associated with the preparation of whey from whole

milk), and determining the inorganic phosphate in the whey by the Lange and Miethke modification (2) of the well-known colorimetric molybdate method. In later work a few samples of serum for comparison were prepared by dialysis of 300 c.c. milk against 6 c.c. water for 48 hours at 0°–5° C., a procedure essentially that recently recommended by Lamphitt and Bushill (1) as the most accurate for the soluble phosphate of milk. In these the phosphate was also determined colorimetrically, and corrected for the dilution caused by dialysis.

The results of the two series are given in Table 1. On the whole agreement is good, the "calcium chloride titration" giving values fairly close to, but somewhat higher than, those of the whey, and apparently still closer to those of the dialysate.

TABLE 1.

Soluble inorganic phosphate in mg. P per 100 *c.c.*

$CaCl_2$ titrn.	Rennet Whey.	$CaCl_2$ titrn.	Dialysate.
36·6	33·5	36·0	36·6
37·8	37·2	36·9	36·6
38·7	35·3	32·2	31·9
38·8	38·1	45·3	45·9
34·7	32·6	33·2	31·6
39·7	35·0		
36·0	34·1		
36·9	33·2		
32·2	31·9		
Av. 36·9	34·7	Av. 36·6	36·3

This difference raised at once the question whether the rennet whey phosphate was a reliable guide to that present in the original milk in soluble condition, and suggested the making of a further series of comparisons between the phosphate content of rennet whey[1] and milk dialysate, using the same (colorimetric) technique for both.

[1] A slight modification was necessary for whey, as the removal of nitrogenous matter by trichloracetic acid in the usual way was found to be incomplete, and the amount remaining in solution was sufficient to upset the colorimetric determination. This difficulty was overcome by the intermediate separation from the trichloracetic serum of the phosphate as magnesium ammonium phosphate thus: 2 c.c. 10 per cent. ammonia, 2 c.c. magnesia mixture, and 2·5 c.c. trichloracetic filtrate were placed in a small centrifuge tube, mixed, allowed to stand overnight, and centrifuged next day at 3,000 r.p.m. for ten minutes. The supernatant liquid was decanted as completely as possible through a glass filter, which was then washed with a few c.cs. of dilute ammonia; after which any of the precipitate which had passed into the filter was washed through with the acid molydate reagent into the original centrifuge tube, and the phosphate content of the tube then estimated colorimetrically as usual.

Typical results are given in Table 2, and show, as was suspected from Table 1, that the whey phosphate is slightly, but definitely, low (average, 6 per cent.).

TABLE 2.

Soluble inorganic phosphate as mg. P per 100 *c.c.*

	Rennet whey.	Dialysate.
	36·0	36·6
	21·1	23·6
	35·3	38·1
	34·1	36·6
	33·2	36·6
	29·8	31·9
	43·1	45·9
	30·4	31·6
Average	32·9	35·1

Since the "calcium chloride" values are on the average about 6 per cent. higher than those for whey (Table 1), and the latter about the same amount (6–7 per cent.) lower than those for the dialysate (Table 2), the agreement between the milk titration and the true soluble phosphate is very close. The volumes of milk and of whey used in Table 1 are not, however, strictly comparable, owing to a volume contraction of about 2 per cent. on preparing rennet whey from milk. Taking this into account, the "calcium chloride" titration will give values about 1–2 per cent. higher, on the average, than the true soluble phosphate. With this correction the method suggested may be regarded as a convenient and reasonably accurate one for the determination of soluble phosphate.

Total Phosphate.

This estimation follows on that of soluble phosphate, and is based on the view already referred to under (*c*), viz. that the colloidal phosphate of milk is largely if not entirely the tertiary calcium salt (which is also of course the state of the formerly soluble phosphate at this stage, as a result of the calcium chloride treatment), and is all convertible into K_3PO_4 by a moderate excess of soluble oxalate, and that the shift of reaction induced by the action of the oxalate on other milk constituents, and in particular on calcium caseinate, is slight, and may be either neglected or allowed for by a small average correction.

On the first of these points the writer and others have already offered evidence (3, 4), the second has been verified experimentally as regards insoluble tricalcium phosphate with pure phosphate solutions (and the results may be

expected to apply equally to this substance in colloidal form), while the third—the effect of oxalate on the reaction of calcium caseinate solution—has been studied for 3 per cent. solutions previously treated as in the "calcium chloride titration" technique, and been found to involve a correction of not more than about 0·05 c.c. N/10 developed alkalinity for 10 c.c. milk. It remains, of course, to verify the general correctness of these statements taken together, by comparing the total inorganic phosphate obtained by this "oxalate titration" with that reached by the customary accurate methods. In the present instance the colorimetric determination of total acid-soluble inorganic phosphate according to the technique of Lange and Miethke (*loc. cit.*) was employed for this purpose.

TABLE 3.

Total inorganic phosphate as mg. P per 100 *c.c.*

	Oxalate titrn.	Acid-soluble.
	62·6	62·0
	63·6	61·3
	62·3	64·8
	63·6	64·2
	63·3	63·6
	68·2	71·0
	63·3	64·5
	67·0	70·7
	65·4	66·0
	60·1	58·6
	67·3	67·6
	69·1	67·0
	66·7	67·6
	78·4	80·9
	57·0	56·4
Average	65·0	65·8

Table 3 gives some results for the comparison. The agreement is very good—in fact closer than expected, as it was anticipated that the slight development of alkalinity which, as was mentioned, arises from the action of the oxalate on the caseinate might have rendered the oxalate titration values slightly the higher. Perhaps this expected correction is compensated for in practice by the presence of a little magnesium phosphate or of a small amount of dicalcium phosphate in the colloidal phosphate, a possibility not excluded by the writer in dealing elsewhere with the constitution of this colloid (*loc. cit.*).

SUMMARY.

A convenient direct titrametric method for the approximate estimation of both soluble and insoluble inorganic phosphates in milk is described. The procedure depends essentially on the precipitation of soluble phosphate as the tricalcic salt by the addition of calcium chloride under controlled conditions, and the subsequent conversion into soluble form of all the inorganic phosphate by the addition of an oxalate. Values for comparative estimations by this and other methods are given, and illustrate the degree of accuracy obtainable.

Incidentally, the inorganic phosphate content of rennet whey was found to be slightly lower (by about 6 per cent. on the average) than that of the dialysate from the same milk.

REFERENCES.

1. LAMPHETT and BUSHILL (1934).—Biochem. J., **28**, 1305.
2. LANGE and MIETHKE (1933).—Milchwirtschaftl. Forsch., **14**, 195.
3. PYNE (1934).—Biochem. J., **28**, 940.
4. PYNE and RYAN (1932).—Sci. Proc. Roy. Dub. Soc., **20** (N.S.), No. 31, 471.
5. RYAN and PYNE (1934).—Sci. Proc. Roy. Dub. Soc., **21** (N.S.), No. 11, 113.
6. SOMMER and MENOS (1931).—J. Dairy Sci., **14**, 136.
7. VAN SLYKE and BOSWORTH (1914).—New York Agr. Exp. Sta. Rep.—Tech. Bulletin No. 37.

No. 25.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1934.

[Read MAY 28. Published separately JULY 15, 1935.]

THERE was a slight increase in the number of batches of radon tubes issued, 311 batches being sent out, as compared with 306 in 1933. On the other hand, the continued tendency towards the use of smaller doses has reduced the total quantity of radon issued to 11,744 millicuries, as compared with 12,996 millicuries in 1933.

No important changes have been made in the technique of preparation of the tubes. Mr. O. FitzGerald, B.SC., and Mr. G. M. MacFarlane, B.SC., were elected to the Radium Exhibitions falling vacant during the year.

Reports from some of the largest users of radon are printed below. In addition to these, returns of the numbers of cases treated with radon, either alone or in conjunction with Surgery or X-Rays, have been received from the following hospitals:—

Rotunda Hospital, Dublin	6 cases.
Central Hospital, Galway	3 „

The total number of cases recorded in these reports is—

Malignant	441
Non-Malignant	149
Total	590

This is a considerable increase on the number (466) recorded in the Report for 1933.

Dr. Chance's report includes a record of the later history of some cases of malignant diseases treated in recent years.

REPORT BY OLIVER CHANCE, M.B., B.CH., D.M.R.E.,
Richmond, Sir P. Dun's, Dr. Steevens', and Coombe Hospitals.

Notes.

About the same number of cases were treated in 1934 as in previous years. Of these, a large number were far advanced. On the whole, however, cancer cases are attending hospital at an earlier stage than half-a-dozen years ago. This is probably due to better co-operation between the general practitioner and the radio-therapist, rather than to improvement in the cancer education of the public.

No changes of importance were made in technique during the past year. The recent publications of Murdoch and Paterson have considerably simplified the physical aspect of surface therapy. It has been used much more than formerly in the treatment of growths of the skin, calculations being based on the graphs prepared by Paterson and Parker.

In the treatment of inter-buccal growths more attention is being paid to oral hygiene than in previous years. If the mouth be a septic one, a dental toilet is done, and mouth washing carried out several times daily for from seven to ten days. During this time the primary growth and glandular areas are usually treated with X-rays through two lateral fields. As a result of this the intra-oral picture often improves in a surprising manner. When radium is later applied the absorption of X-rays is, of course, allowed for, and the time of radium application shortened accordingly.

At the end of this year's Report tables are given for cases of cancer of the lip and oral carcinoma treated during the past four years, and of cancer of the cervix uteri for the five years, 1930 to 1934. All cases untraced or dead are regarded as having died of cancer, although in at least two cases the cause of death is known to have been unrelated to malignant disease. It is also probable that a small proportion of the untraced cases are still alive and free from disease.

List of Cases Treated with Radium or Radon (Total, 212).

Malignant, 156.

Rodent Ulcer	54	Carcinoma Vulva	1
Carcinoma Skin	12	„ Breast	1
„ Lip	22	„ Thyroid	1
„ Tongue	26	„ Anus	1
„ Cheek	1	„ Bladder	1
„ Palate	5	„ Prostate	1
„ Floor of Mouth ...	5	Branchial Carcinoma	3
„ Gum	3	Sarcoma	1
„ Cervix Uteri ...	18		

Non-Malignant, 56.

Tubercular Peritonitis ...	20	Keloids	5
Toxic Goitre	11	Naevi	12
Painful Scars	2	Lupus	2
Warts	2	Menopausal Hæmorrhage ...	2

Malignant Cases.

(Including also some cases treated with X-rays, etc.)

Rodent Ulcers, 59.

Treated by surgery, 5. Treated with radium, 54. Well, 38. Improved, 1. Unimproved, 1. Untraced, 9. Still under treatment, 4. Refused treatment, 1.

Carcinoma of Skin, 15.

Treated by surgery, 2. Unsuitable, 1. Treated with radium, 12. Well, 9. Still under treatment, 1. Untraced, 1. Dead, 1.

Buccal Carcinoma, 62.

Cases seen, 86. Treated, 62. Unsuitable, 24.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Still under Treatment.	Dead.
Carcinoma Lip, 22.							
Group 1	15	11	1	1	2	—	—
„ 2	2	1	1	—	—	—	—
„ 3	5	—	1	3	—	1	—
Carcinoma Tongue, 26.							
Group 1	16	8	2	2	2	—	2
„ 2	5	2	1	—	—	—	2
„ 3	5	—	2	2	—	—	1
Carcinoma Cheek, 1.							
Group 1	1	1	—	—	—	—	—
Carcinoma Palate, 5.							
Group 1	5	3	—	1	—	—	1
Carcinoma Floor of Mouth, 5.							
Group 1	3	3	—	—	—	—	—
„ 2	1	—	1	—	—	—	—
„ 3	1	—	1	—	—	—	—
Carcinoma Gum, 3.							
Group 1	2	1	—	1	—	—	—
„ 2	1	—	—	—	—	—	1

Carcinoma Cervix Uteri.

Cases seen, 20. Unsuitable, 2. Treated, 18.

	No. of cases.	Well.	Improved, but disease still active.	Unimproved.	Untraced.	Dead.
Operable ...	4	3	—	—	1	—
Borderline ...	6	2	3	—	—	1
Inoperable ...	8	3	1	1	1	2

Carcinoma Body of Uterus, 4.

Unsuitable, 2. Treated with X-rays and surgery, 3. Treated with X-rays only, 1. Well, 2. Untraced, 1. Dead, 1.

Carcinoma Vagina, 0.

Unsuitable, 1.

Carcinoma Vulva, 1.

Treated with radium.—Well, 1.

Carcinoma Breast, 15.

	No. of cases.	Free from disease.	Unimpr.	Dead.
Prophylactic X-rays ...	6	6	—	—
Primary Irradiation (Radium) ...	1	1	—	—
Post-Operative Recurrence ...	3	—	2	1
Palliative X-rays ...	5	—	—	—

Carcinoma Thyroid, 1.

Unsuitable, 2. Treated with radium and X-rays, 1. Improved, 1.

Carcinoma Parotid, 0.

Unsuitable, 1.

Carcinoma Anus, 1.

Treated, 1. Well, 1.

Carcinoma Bladder, 1.

Treated, 1. Dead, 1.

Carcinoma Prostate, 1.

Treated, 1. Improved, 1.

Branchial Carcinoma, 3.

Treated, 3. Improved, 2.

Carcinoma Rectum, 2.

Unsuitable, 3. Treated with X-rays, 2. Improved, 1. Dead, 1.

Sarcoma, 2.

Unsuitable, 4. Treated, 2. Sarcoma jaw treated by surgery and radium—well, 1. Sarcoma ovary treated with X-rays—unimproved, 1.

Carcinoma Maxilla, 0.

Unsuitable, 2.

Intrinsic Carcinoma Larynx, 2.

Treated with X-rays, 2. Well, 1. Dead, 1.

Post-Cricoid Carcinoma, 5.

Treated with X-rays, 5. Well, 1. Temporarily improved, 3. Dead, 1. Unsuitable, 1.

Carcinoma Pharynx, 1.
Unsuitable, 2. Treated with X-rays, 1. Unimproved, 1.

Carcinoma Oesophagus, 1.
Treated with X-rays, 1. Dead, 1.

Hodgkin's Disease, 2.
Treated with X-rays, 2. Improved, 2.

Non-Malignant Cases.

(Including some treated with X-rays.)

Tubercular Peritonitis, 20.
Well, 8. Improved, 2. Untraced, 6. Unimproved, 2. Dead, 2.

Toxic Goitre, 11.
Unsuitable, 1. Well, 2. Unimproved, 2. Improved, 2. Still under treatment, 5.

Adenoma Prostate, 3.
Treated with X-rays, 3. Well, 1. Greatly improved, 2.

Painful Scars, 2.
Treated, 2. Unimproved, 2.

Warts, 2.
Treated, 2. Well, 2.

Keloids, 5.
Treated, 5. Well, 2. Improved, 2. Unimproved, 1.

Naevi, 12.
(*a*) Angiomata, 9.
Well, 5. Still under treatment, 3. Untraced, 1.
(*b*) Cavernous Naevi, 3.
Improved, 3.

Furunculosis, 2.
Treated with X-rays and healed without incision, 2.

Lupus, 2.
Well, 1. Disease still active, 1.

Splenic Anæmia, 1.
Treated with X-rays, 1. Improved, 1.

Lymphatic Leucæmia, 1.
Treated with X-rays, 1. Improved, 1.

Menopausal Hæmorrhage, 2.
Well, 2.

Records of Cases Treated in Recent Years.

Cancer of Lip.

Stage 1.						*Stage* 2.					
		Alive years after treatment.						Alive years after treatment.			
Year.	Total treated.	1	2	3	4	Year.	Total treated.	1	2	3	4
1931.	8	6	5	5	5	1931.	3	1	0	0	0
1932.	11	7	6	6		1932.	4	2	1	1	
1933.	8	5	5			1933.	4	2	2		
1934.	15	11				1934.	2	1			

Cancer of Tongue.

Stage 1.						*Stage* 2.					
		Alive years after treatment.						Alive years after treatment.			
Year.	Total treated.	1	2	3	4	Year.	Total treated.	1	2	3	4
1931.	11	6	4	4	3	1931.	9	2	2	1	1
1932.	13	5	4	2		1932.	7	2	0	0	
1933.	13	8	6			1933.	5	2	0		
1934.	16	8				1934.	5	2			

Cancer of Cheek.

Stage 1.						*Stage* 2.					
		Alive years after treatment.						Alive years after treatment.			
Year.	Total treated.	1	2	3	4	Year.	Total treated.	1	2	3	4
1931.	3	1	1	1	1	1931.	1	0	0	0	0
1932.	3	2	2	1		1932.	–	–	–	–	–
1933.	1	1	1			1933.	1	1	1		
1934.	1	1				1934.	–	–			

Cancer of Gum.

Stage 1.						*Stage* 2.					
		Alive years after treatment.						Alive years after treatment.			
Year.	Total treated.	1	2	3	4	Year.	Total treated.	1	2	3	4
1931.	2	1	1	0	0	1931.	1	0	0	0	0
1932.	5	2	2	2		1932.	–	–	–	–	
1933.	2	1	1			1933.	–	–	–		
1934.	2	1				1934.	1	0			

Cancer of Palate.

Stage 1.						*Stage* 2.					
		Alive years after treatment.						Alive years after treatment.			
Year.	Total treated.	1	2	3	4	Year.	Total treated.	1	2	3	4
1931.	3	3	2	2	2	1931.	–	–	–	–	–
1932.	5	3	3	2		1932.	1	0	0	0	
1933.	4	2	1			1933.	1	0	0		
1934.	5	3				1934.	–	–			

Cancer, Floor of Mouth.

Stage 1.

Year.	Total treated.	Alive years after treatment. 1	2	3	4
1931.	5	2	1	1	1
1932.	5	3	1	1	
1933.	5	4	1		
1934.	3	3			

Stage 2.

Year.	Total treated.	Alive years after treatment. 1	2	3	4
1931.	2	0	0	0	0
1932.	1	0	0	0	
1933.	2	1	1		
1934.	1	1			

Cancer of Fauces.

Stage 1.

Year.	Total treated.	Alive years after treatment. 1	2	3	4
1931.	3	1	0	0	0
1932.	3	2	1	1	
1933.	3	1	1		
1934.	–	–			

Stage 2.

Year.	Total treated.	Alive years after treatment. 1	2	3	4
1931.	–	–	–	–	–
1932.	4	1	0	0	
1933.	4	1	1		
1934.	3	1			

Cancer of Cervix Uteri.

Stage 1.

Year.	Total treated.	Alive years after treatment. 1	2	3	4	5
1930.	3	3	2	1	1	1
1931.	5	3	3	2	2	
1932.	4	4	1	1		
1933.	1	1	1			
1934.	4	3				

Stage 2.

Year.	Total treated.	Alive years after treatment. 1	2	3	4	5
1930.	5	3	2	1	1	1
1931.	6	4	2	1	1	
1932.	3	2	0	0		
1933.	3	2	1			
1934.	6	5				

Stage 3.

Year.	Total treated.	Alive years after treatment. 1	2	3	4	5
1930.	3	2	1	0	0	0
1931.	8	3	1	0	0	
1932.	6	1	0	0		
1933.	3	2	0			
1934.	8	4				

Report by W. G. Harvey, M.D., F.R.C.P.I.,

Adelaide Hospital, Dublin.

Cases treated with Radium Emanation at the Adelaide Hospital during 1934.

(Each case received approximately 200 mgm.-hour per sq. cm. of growth.)

1. A.H., 25.1.34.—*Squamous Carcinoma of Nose.* Previously treated with X-rays, with only temporary healing. Result—apparent cure.

2. Mrs. McA., 15.3.34.—*Squamous Carcinoma.* Patient did not return or reply to letter, but we believe that the tumour has disappeared. She had previously been treated with X-rays without permanent result.

3. H.R., 29.5.34.—*Rodent Ulcer.* Also previously treated with X-rays with temporary benefit. Remained a thoroughly sound scar when last seen this year.

4. R.D., 4.10.34.—*Epitheliomatous Ulcer of Hand..* The raised edges have completely disappeared. There is still ulceration and pain. Report of a subsequent section stated inflammatory only.

Report by Oswald J. Murphy, M.B., B.Ch.,

St. Vincent's Hospital, Dublin.

1934.

List of Cases Treated (Total, 191).

Malignant Cases, 113.

	Number seen.	Number Treated.	Died.	Untraced.	Under Treatment.	Showing no Active Signs.
Carcinoma.						
Lip	8	7	2	–	3	2
Tongue	7	5	3	–	1	1
Palate	1	1	–	–	1	–
Tonsil	2	2	–	–	1	1
Pharynx	1	1	–	–	1	–
Larynx	2	1	–	–	1	–
Breast	2	2	1	–	1	–
„ Secondaries ...	12	12	3	3	6	–
Anus	1	1	–	–	–	1
Uterine Cervix ...	3	3	–	1	–	2
„ Body ...	1	1	–	1	–	–
Skin Surfaces ...	31	28	–	6	10	12
Glands in Neck ...	7	7	2	1	4	–
„ „ Axilla ...	2	2	–	–	2	–
Rodent Ulcer ...	38	38	–	4	10	24
Sarcoma Maxillary Antrum ...	2	2	–	–	2	–
	120	113	11	16	43	43

Non-Malignant Cases, 78.

	Treated.	Under Treatment.	Died.	Improved.	Well.
Exophthalmic Goitre	1	1	–	1	–
Enlarged Spleen	1	1	–	–	–
Hodgkin	9	2	2	5	2
T.B. Cervical Glands	12	4	–	8	4
Angiomata	14	4	–	4	10
Lupus Vulgaris	2	2	–	2	–
Lupus Erythematosus	1	1	–	1	–
Keloids	7	2	–	7	–
Warts	29	3	–	8	21
Corns	2	1	–	1	1
	78	21	2	37	38

During the year the techniques employed have been altered only slightly.

Report from St. Anne's Hospital.

Radiologist—Dr. Oliver Chance.

Many more cases were treated in 1934 than in previous years. During the year a small quantity of radium in the form of low intensity needles was purchased. A new deep X-ray therapy plant was also installed in the place of the old one, which had become obsolete. As a result of these additions it became possible to give palliative treatment to patients whom formerly one was obliged to turn away.

The tables given below show that after their discharge from hospital about 25 per cent. of the patients could not be traced. Not only did they fail to return to hospital for inspection or further treatment, but most of them did not even bother to answer letters of enquiry. While lack of co-operation on the part of the patients is partly to blame for this unsatisfactory state of affairs, the cumbersome methods of administration adopted by local authorities is the chief cause. Until some satisfactory "follow up" system—such as that in use in Sweden, where much less than 1 per cent. of patients are untraced—is evolved, the treatment of cancer patients in Ireland must remain unsatisfactory.

Malignant Cases, 159.

Rodent Ulcers.

Total number of cases seen, 45. Treated, 40. Well, 23. Improved, 5. Untraced, 10. Unimproved, 1. Refused treatment, 1. Unsuitable, 5.

Carcinoma of Skin.

Total number of cases seen, 25. Treated, 20. Refused treatment, 1. Unsuitable, 4.

Buccal Carcinoma, 62.

Total number of cases seen, 69. Treated, 57. Unsuitable, 12.

	No. of cases.	Well.	Primary well, but glands involved.	Under treatment.	Imp.	Unimp.	Untraced.	Dead.
Carcinoma Lip, 21; *Unsuitable*, 2.								
Group 1	9	2	—	—	3	—	4	—
„ 2	4	—	—	—	3	—	—	1
„ 3	6	—	1	—	1	—	4	—
Carcinoma Tongue, 22; *Unsuitable*, 4.								
Group 1	7	4	—	—	1	—	2	—
„ 2	6	3	—	1	—	—	—	2
„ 3	5	—	—	—	4	—	1	—
Carcinoma Cheek, 4.								
Group 1	2	2	—	—	—	—	—	—
„ 2	—	—	—	—	—	—	—	—
„ 3	2	—	—	—	2	—	—	—
Carcinoma of Palate, 5; *Unsuitable*, 2.								
Group 1	1	1	—	—	—	—	—	—
„ 2	—	—	—	—	—	—	—	—
„ 3	2	—	—	—	1	—	1	—
Carcinoma of Floor of Mouth, 7; *Unsuitable*, 1.								
Group 1	4	2	1	—	—	1	—	—
„ 2	1	—	—	—	—	—	1	—
„ 3	1	—	—	—	1	—	—	—
Carcinoma of Gum, 7; *Unsuitable*, 2.								
Group 1	1	—	1	—	—	—	—	—
„ 2	1	—	—	—	—	—	1	—
„ 3	3	—	—	—	2	—	1	—
Carcinoma Fauces, 3; *Unsuitable*, 1.								
Group 1	—	—	—	—	—	—	—	—
„ 2	—	—	—	—	—	—	—	—
„ 3	2	—	—	—	2	—	—	—

Carcinoma of Cervix Uteri.

Number of cases seen, 2. Treated, 1. Unsuitable, 1. Inoperable, 1. Untraced, 1.

Carcinoma of Body of Uterus.

Number of cases seen, 4. Treated, 4. Well, 1. Untraced, 3.

Carcinoma of Breast.

Number of cases seen, 18. Treated, 12. Transferred for treatment, 1. Unsuitable, 5.
Prophylactic irradiation, 4. Free from disease, 4.
Palliative X-ray, 3. Improved, 3.
Pre-operative X-ray, 2.
Recurrent cases, 1. Improved, 1.
Treated with surgery, 2. Improved, 1. Dead, 1.
Treated with Radium, 1. Well, 1.

Carcinoma of Penis.

Cases seen, 2. Treated, 1. Refused treatment, 1. Well, 1.

Carcinoma of Bladder.

Cases seen, 2. Treated, 2. Improved, 1. Dead, 1.

Carcinoma of Anus.

Cases seen, 2. Treated, 2. Untraced, 1. Dead, 1.

Carcinoma of Rectum.

Cases seen, 9. Treated, 7. Unsuitable, 1. Transferred for treatment, 1. Relieved, 4. Died, 1.

Carcinoma of Vulva.

Cases seen, 3. Treated, 1. Unsuitable, 2. Improved, 1.

Carcinoma of Thyroid.

Treated, 1. Unimproved, 1.

Carcinoma of Larynx.

Cases seen, 9. Treated, 5. Unsuitable, 4. Temporarily relieved, 3. Died, 4.

Carcinoma of Trachea.

Unsuitable, 1. Died, 1.

Carcinoma of Oesophagus.

Cases seen, 3. Treated, 1. Unsuitable, 2. Improved, 2.

Primary Carcinoma of Glands of Neck.

Cases seen, 4. Treated, 2. Unsuitable, 2. Relieved, 1. Untraced, 1.

Carcinoma of Mandible.

Treated, 1. Improved, 1.

Carcinoma of Maxilla.

Treated, 1. Died, 1.

Carcinoma of Chest Wall.

Unsuitable, 1.

Carcinoma of Liver.

Unsuitable, 1.

Carcinoma of Stomach.
Unsuitable, 2.

Sarcoma of Mandible.
Treated, 1. Temporarily relieved, 1.

Non-Malignant Cases, 15.

Tubercular Peritonitis ...	1	Papilloma of Naevi	8
Papilloma of Tongue	1	Myelogenous Leukæmia ...	1
„ Lip	1	Hodgkin's Disease	1
„ Rectum	2		

No. 26.

NOTE ON THE EFFECT OF STORAGE ON THE COLOUR AND ON THE FREE FATTY ACID CONTENT OF A COMMERCIAL SAMPLE OF VETERINARY COD-LIVER OIL.

By E. J. SHEEHY, F.R.C.Sc.I., B.Sc.,
University College, Dublin.

[Read May 28. Published separately July 16, 1935.]

An earlier investigation (1) into the effect of the storage of cod-liver oil on its vitamin D content indicated, *inter alia*, the possibility of colour changes, and of an increase in the acid content of oil stored for a considerable period. Free fatty acids are toxic to the body, and are particularly injurious to young animals or birds, and it is essential that storage conditions of cod-liver oil should be such as to inhibit changes therein which would lead to an increase in the free acids. The appearance of colour other than "water white" or amber in cod-liver oil is correlated with the presence of deleterious material, and, as in the case of the free acids, knowledge of the conditions of storage which are unfavourable to change in colour is important.

Exposure to light has a bleaching effect (2), and reduces the vitamin A content, so that it is recommended to store cod-liver oil in dark glass rather than clear bottles.

To test the effect of storing under various conditions, a five-gallon drum of veterinary cod-liver oil, which was procured from a reputable firm, was divided into a number of parts, which were kept for 20 months under different conditions, as described below:

Sample 1, in a clear glass wide-mouthed jar filled to the top and sealed to exclude air.

Sample 2, in a clear glass wide-mouthed jar filled to the top but not stoppered, so that air had access to the oil.

Sample 3, in a tin container filled to the top and sealed to exclude air.

Sample 4, in a clear glass wide-mouthed jar filled to the top and sealed to exclude air: some thoroughly washed and steamed oak shavings were placed in the bottle before sealing.

Sample 5, in a clear glass wide-mouthed jar filled to the top and sealed to exclude air: a strip of rusty iron was placed in the bottle before sealing.

Sample 6, in the original container, which was made of sheet iron: the vessel was about one-third full but was stoppered.

All the samples were placed in a dark cupboard, and undisturbed during storage.

After 20 months they were examined for colour and for free fatty acid content, and the following table gives the results. For comparative purposes similar particulars for the oil prior to storage are given :—

	Colour and Appearance.	Free Fatty Acid (as oleic acid).
Original oil,	amber—clear ...	1·54 per cent.
Sample 1,	amber—clear ...	1·68 „
„ 2,	orange—clear ...	3·20 „
„ 3,	amber—clear ...	1·76 „
„ 4,	olive tinge—clear ...	1·75 „
„ 5,	light orange—slightly turbid	1·71 „
„ 6,	deep orange red—very turbid	2·43 „

The outstanding feature of the results is the very considerable increase in free fatty acid in the samples to which air had access, namely, samples 2 and 6. While number 2 was freely exposed to atmospheric air, the aeration of number 6 was limited to that quantity of air which was enclosed in the vessel with the sample. Oil stored in a similar manner to number 6 was found by Holmes, Tripp and Menard (3) to increase in free fatty acid content. Apparently, in the case of samples 2 and 6, a considerable amount of oxidation had gone on, with consequent increase in free acids. In the remainder of the samples a very slight increase had taken place in free acid content, this being due presumably to hydrolytic changes.

Comparison of samples 1 and 2 and of 5 and 6 shows that a change in colour of the oil occurred, which may be correlated with change in free acid content, so that air should be excluded from stored cod-liver oil, not alone to prevent an increase in the acids, but also to prevent the development of undesirable colour changes.

The results also show that the colour and appearance of stored oil may be affected by contact with the material of the container. Thus, fresh oak wood —sample 4—imparts a definite tinge, which has no relationship with the development of acid. Similarly, iron may impart an undesirable colour, and in addition, presumably because of the inter-action of the acids of the oil with the metal, imparts a turbidity to the oil, which, however, disappears through sedimentation of the solid particles when the oil is allowed to stand for a considerable period. The degree of turbidity is related to the acid content of the oil; hence the importance, again in this connection, of maintaining at a low level the free fatty acids of cod-liver oil.

Summary.

1. A clear amber-coloured oil containing 1½ per cent. of free fatty acid was preserved under different conditions for a period of 20 months.

2. A very slight increase in the free fatty acid content occurred in those samples from which air was excluded.

3. The free fatty acids of those samples to which air had access increased appreciably.

4. Associated with the increase in free fatty acid there occurred a colour change.

5. The results also indicate the possibility of a development of colour arising from contact of the oil with the container: fresh oak wood or iron may be responsible for this effect, and the latter may also cause turbidity of the oil.

References.

1. Sheehy.—Sc. Proc. Roy. Dub. Soc., **20** (N.S.), p. 463 (1932).
2. Drummond and Hilditch.—Empire Marketing Board. Publication No. 35 (1930).
3. Holmes, Tripp and Menard.—"Oil and Soap," **11**, 102 (1934).

SCIENTIFIC PROCEEDINGS.

VOLUME 21.

1. Some Measurements of the Brightness of Various Parts of the Sky by means of a Rectifier Photo-Electric Cell. By H. H. POOLE, SC.D., and W. R. G. ATKINS, SC.D., F.INST.P., F.R.S. 6d. (December, 1933.)
2. —Some Difficulties in Current Views of the Thermal History of the Earth. By J. H. J. POOLE, SC.D. 1s. (January, 1934.)
3. —Abnormal Cones of *Fitzroya* and their Bearing on the Nature of the Conifer Strobilus. By JOSEPH DOYLE, Botany Department, University College, Dublin, and MARY O'LEARY, Assistant in the Department. (Plate 1.) 1s. 6d. (February, 1934.)
4. —A Note on the Molecular Complexity of Amylose in Potato Starch. By J. REILLY, M.A., SC.D., P. P. O'DONOVAN, PH.D., and MISS H. MURPHY, M.SC., University College, Cork. 6d. (February, 1934.)
5. —The Oxidation of Hydrazine by Potassium Ferricyanide. Part I: The Influence of Gaseous Supersaturation on the Measurement of Reaction Velocity. Part II: The Reaction in Presence of Acetone. By THOMAS NORMAN RICHARDSON, M.SC., and KENNETH CLAUDE BAILEY, SC.D., LITT.D., Fellow of Trinity College, Dublin. 1s. (February, 1934.)

[Nos. 1 to 5, price 4s.]

6. —A Convenient Method of Measuring Resistances of the Order of 10^{12} Ohms with a Sensitive Ballistic Galvanometer. By J. H. J. POOLE, SC.D. 6d. (June, 1934.)
7. —Report of the Irish Radium Committee for the Year 1933. (Including Reports by Oliver Chance, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', and Coombe Hospitals; W. G. Harvey, M.D., F.R.C.P.I., Adelaide Hospital; W. J. MacHugh, M.B., B.CH., B.A.O., D.M.R.E., Galway Central Hospital; Oswald J. Murphy, M.B., B.CH., St. Vincent's Hospital, Dublin.) 6d. (July, 1934.)
8. —The Chemical Constituents of Lichens found in Ireland. *Buellia canescens.*—Part I. By T. J. NOLAN, B.A., D.SC., University College, Dublin. (Plate 2.) 1s. (July, 1934.)
9. —Derangement of the Digestive Processes in the Milk-fed Calf due to abnormal Curd formation in the Fourth Stomach. By E. J. SHEEHY, B.SC., F.R.C.SC.I., University College, Dublin. 1s. (July, 1934.)
10. The Approximate Determination of the Vibration of Beams and the Whirling of Shafts. By H. H. JEFFCOTT, SC.D. 2s. 6d. (August, 1934.)
11. —Investigations of the Cryoscopy of Milk. By J. J. RYAN and G. T. PYNE (Department of Dairy Chemistry, University College, Cork.) [Communicated by Professor J. Reilly.] 6d. (July, 1934.)
12. The Influence of the Stage of Lactation on Fat Estimations by the Gerber Method. By J. LYONS and M. O'SHEA, Dairy Technology Department, University College, Cork. [Communicated by Mr. E. J. Sheehy.] 1s. (August, 1934.)

[Nos. 6 to 12, price 6s.]

13. —The Measurement of the Current Generated by Rectifier Photo-Cells. By H. H. POOLE and W. R. G. ATKINS, F.R.S. 6d. (November, 1934.)
14. —The Chemical Constituents of Lichens Found in Ireland. *Lecanora Gangaleoides.*—Part I. By JOSEPHINE HARDIMAN, M.SC.,; J. KEANE, PH.D.; and T. J. NOLAN, B.A., D.SC., University College, Dublin. 6d. (January, 1935.)
15. —The Cellulose of Marine Algae.—By THOMAS DILLON, M.A., D.SC., and TADHG O'TUAMA, M.SC., University College, Galway. 6d. (January, 1935.)
16. —The Thermal Decomposition of Hydrogen Peroxide in Presence of Glass Wool and Copper Sulphate. By KENNETH C. BAILEY, SC.D., LITT.D., Fellow of Trinity College, Dublin. 1s. (January, 1935.)
17. —On the Preparation and Properties of Alginic Acid and on the Extraction of Marine Algae with Various Solvents (Preliminary Note). By VINCENT BARRY, M.SC., and THOMAS DILLON, M.A., D.SC., University College, Galway. 6d. (January, 1935.)
18. —A Crate for the Collection of Fæces and Urine Adjustable for Metabolism (Solid and Liquid) Experiments with Pigs, Sheep, and Cattle of Various Sizes. By E. J. SHEEHY, F.R.C.SC.I., B.SC., Department of Animal Nutrition, University College, Dublin. 6d. (January, 1935.)
19. —Pollination in *Saxegothaea.* By JOSEPH DOYLE, Botany Department, University College, Dublin, and MARY O'LEARY, National Museum, Dublin. (February, 1935.)
20. —Pollination in *Pinus.* By JOSEPH DOYLE, Botany Department, University College, Dublin, and MARY O'LEARY, National Museum, Dublin. (Plate 3.) (February, 1935.)
21. —Pollination in *Tsuga, Cedrus, Pseudotsuga,* and *Larix.* By JOSEPH DOYLE, Botany Department, University College, Dublin, and MARY O'LEARY, National Museum, Dublin. (Plates 4 and 5.) (February, 1935.) Nos. 19 to 21, 4s.

[Nos. 13 to 21, price 6s.]

22.—Investigations on the Control of Seedling Diseases of Sugar Beet. By WILLIAM HUGHES, B.AGR.SC., M.SC., Albert Agricultural College, Glasnevin, Dublin. [Communicated by Dr. P. A. Murphy.] 6d. (April, 1935.)

23.—On the Characteristics of *Bacterium Violaceum* (Schröter) and Some Allied Species of Violet Bacteria. By GEORGE CRUESS-CALLAGHAN, M.SC., and M. J. GORMAN, A.R.C.SC.I. (Plate 6.) 1s. (May, 1935.)

24.—A Simple Titrametric Method for the Approximate Determination of Milk Phosphates. By G. T. PYNE, Department of Dairy Chemistry, University College, Cork. [Communicated by Professor T. J. Nolan.] 6d. (June, 1935.)

25.—Report of the Irish Radium Committee for the year 1934. (Including Reports by Oliver Chance, M.B., B.CH., D.M.R.E., Richmond, Sir Patrick Dun's, Dr. Steevens', Coombe, and St. Anne's Hospitals; W. G. Harvey, M.D., F.R.C.P.I., Adelaide Hospital, Dublin; Oswald J. Murphy, M.B., B.CH., St. Vincent's Hospital, Dublin. 1s. (July, 1935.)

26.—Note on the Effect of Storage on the Colour and on the Free Fatty Acid Content of a Commercial Sample of Veterinary Cod-liver Oil. By E. J. SHEEHY, F.R.C.SC.I., B.SC., University College, Dublin. 6d. (July, 1935.)

[Nos. 22 to 26, price, 3s.]

PRINTED BY PONSONBY AND GIBBS, UNIVERSITY PRESS, DUBLIN.